marked (July 13), two individuals were 22 and 34 inches away from the original location. Sixty days later (September 11), or eleven weeks after the beginning of the experiment, one snail was 62 inches from its original position. Twenty-six days after the other set was marked, (July 30) two individuals were 22 and 28 inches away. Twenty-five days later (Aug. 24) two were 10 and 18 inches away; another eighteen days later (Sept. 11), or nearly eleven weeks after the snails were marked, three were only 6, 7 and 30 inches away respectively.

*T. lapillus,* which feeds principally on *Balanus balanoides* (L.) and *Mytilus edulis* L., showed a tendency to remain on barnacles, in crevices, and under seaweeds for long periods and to remain within a restricted locality. In general it did not travel as much nor as extensively as *L. litorea* which feeds chiefly on algae, both microscopic and macroscopic.

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## LIFE CYCLE OF LYMNAEA STAGNALIS COMPLETED AT ROOM TEMPERATURE WITHOUT ACCESS TO AIR

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It has been known for some time that pulmonate snails may, under certain conditions, become water-breathing. In fact *Planorbis cristatus* is said to have its lung permenently filled with water (Willem, 1895; von Buddenbrock, 1924); and *Planorbis corneus* is reported to have developed accessory gills in its lung cavity (von Buddenbrock, 1924). It has been further claimed by Precht (1939) that *Lymnaea stagnicola* assumes purely cutaneous, aquatic respiration at a temperature of 5° C. or below. According to Cheatum (1934) *Helisoma campanulatum smithii, H. antrosum percarinatum, Lymnaea emarginata angulata* and *Physa sayi crassa* are probably able to complete their life cycles and reproduce normally without coming to the surface for air. Forel and Du Plessis (1874) and Brot (1874) reported *Lymnaea abyssicola* living at depths of 25 to 250 meters in Lake Geneva, Switzerland. When collected, their lung cavities were filled with water and, living at such depths, they could hardly have made contact with the surface at any time during their life history.

Von Siebold (1875) records having seen, in deep water, colonies of *Lymnaea auricularia* which, during the period of his observations, were never observed to visit the surface. Fresh-water pulmonates that spend the winter under the ice are probably forced to depend to a large extent, if not exclusively, on cutaneous respiration. Willem (1896) showed that *Lymnaea* and *Planorbis* could be kept submerged for long periods in well-aerated water, but died in a few hours when immersed in water that had been boiled and protected from contact with air. In the land snail *Helix*, with the shell removed and the body kept submerged in normal saline, cutaneous respiration has been shown to exceed pulmonary respiration (Courtois and Duval, 1927; Raffy and Fischer, 1931). Cheatum (1934) showed that nine species of fresh-water snails can withstand enforced and prolonged submersion (62 days). The percentage of survival was less in warm water (21.6° to 25.6° C.) than in cooler water (11° C.). Removal of the air from the lung cavity likewise reduced the percentage of survival.

H. B. Baker (1912) reported six species of pulmonates from Douglas Lake, Michigan, including *Lymnaea stagnalis perampla* Walker, *L. emarginata angulata* (Sowerby), *Physa ancillaria parkeri* (Currier), *P. bicarinatus portagensis* Baker, *P. b. percarinatus* Walker, and *Planorbis campanulatus smithii* Baker, which according to his observations "appear to be all deep water forms and have apparently become adapted to breathe water instead of air." He suggested the possibility that the water-breathing habit had even affected the body form of some of the species to the extent of modifying the shell type of *Lymnaea stagnalis appressa* toward that of *L. s. perampla* and the shell form of typical *Physa ancillaria* Say toward that of *P. a. parkeri*. Two years later (Baker 1914) he recorded further observations on the water-breathing habits of Douglas Lake snails, in the course of which he noted that the adults of *Physa ancillaria parkeri* "were so completely habituated to breathing water that when placed in small aquaria, they died soon after exhausting the air in the water, without ever attempting to come to the surface to breathe; although when once taught to breathe air, by the simple method of exposing them out of water until the water in their lungs partially evaporated or was otherwise replaced by air, they could be kept in a small dish for several months and would come to the surface regularly like any ordinary, air-breathing form.''

In the course of other studies on the biology of *Lymnaea stagnalis appressa* Say, pursued in this laboratory, it occurred to us that it would be interesting to find out whether this snail, which is normally air-breathing, could be grown through a complete life cycle in the laboratory at room temperatures without ever being permitted to come to the surface for air.

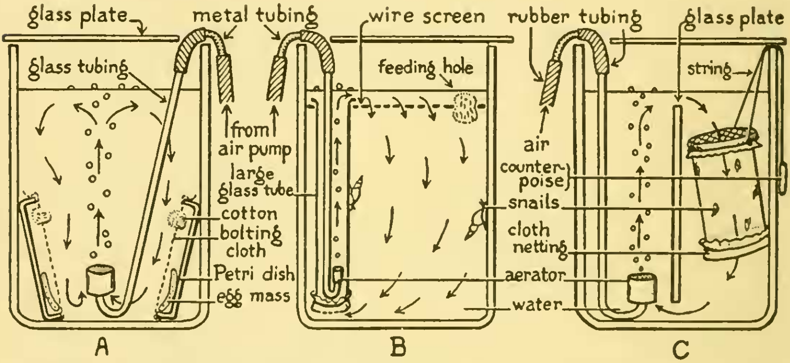


Fig. 1. Diagrams showing containers used to provide the egg masses and growing snails with aerated water while at the same time preventing access to the surface.

Accordingly several newly deposited egg masses were collected, on Feb. 28 and Mar. 1, 1940, and placed in shallow glass containers (one member of a Petri dish pair). Three such dishes were set up, and each was covered with coarse-meshed bolting cloth, in which there was a small feeding hole plugged with cotton that could be removed at feeding times. These dishes were submersed in water in a large glass container kept at room temperature in the laboratory. All air bubbles were carefully removed after immersion. The dishes were placed near an air releaser which maintained a constant current of aerated water past the bolting cloth covers of the dishes, but not so near that any bubbles from the air releaser would come in contact with the cloth (Fig. 1 A).

The room in which the snails were kept had a thermostatically controlled temperature, which was normally near 20° C., except in the summer when a somewhat higher temperature was reached (up to 34.4° C. at one time, as will be mentioned later).

Hatching occurred within a month (Mar. 28 to Apr. 4). The young snails were fed small pieces of tender green lettuce, which had been previously immersed in water and freed of all air bubbles. A sample of water taken from the inside of the Petri dish gave, with the micro-Winkler method (Lund, 1922), a reading of 1.8 cc. dissolved oxygen per liter, as compared with 2.5 to 3.1 cc. in the outside water, indicating that the utilization of oxygen inside the dish was greater than its replacement by the circulation of the water.

On July 12, about three months after hatching, all the snails in one of the dishes died, apparently due to depletion of the oxygen supply past the point of toleration. These snails had shell lengths of 9.0 to 17.5 mm. at the time of death, approximately ten weeks after hatching. In this same period of time, normal snails fed on lettuce and allowed access to air ordinarily reached a shell length of 20 to 30 mm. under the culture methods employed at that time in the laboratory. Evidently, therefore, there was a considerable retardation of growth in spite of the fact that a small excess of food (lettuce) was regularly provided.

After the death of the snails in the one dish, those in the remaining two dishes were transferred to more commodious quarters, a battery jar six inches in diameter and eight inches high, equipped, as shown in Fig. 1 B, to provide for better aeration of the water but, as before, preventing access to the surface. In these and in all other transfers (i.e., when water was changed, at intervals of one to two weeks), care was taken to keep the snails immersed in water at all times during the transfer, so that there would be no possibility of their taking air into their lung cavities at this time. In young snails, the shell is sufficiently transparent to detect the presence of air in the lung, and the snails were regularly checked when the transfers were made to see if air was present. Another check was possible because snails with no air in the lung are considerably heavier than water and sink rapidly when allowed to fall through the water; snails with air in the lung either float or sink rather slowly. At no time was air observed in the lungs of any of the snails except the two that escaped as mentioned below.

On Dec. 26, 1940, nearly nine months after hatching, the first egg mass was found in the jar. Normally under our laboratory cultural conditions, snails start laying about three or four months after hatching. Evidently, therefore, submersion had more than doubled the time required to reach sexual maturity and lay eggs. On Jan. 8, 1941, another egg mass was found, and on Jan. 9, four more. Other egg masses were laid at intervals up to April 11, when four of the snails were still alive. At this time, two of the animals escaped, through an opening caused by an undetected bend in the wire barrier, and took air into their lung cavities. These were discarded, and the two remaining ones retained until their death on May 31, 1941, at an age of approximately 14 months. In our usual laboratory cultures, snails of this species commonly live from 12 to 18 months. At death, the shell length is usually between 40 and 50 mm., as compared with 33 and 34 mm. respectively in the two oldest submersed snails. During most of their adult life, the submersed snails had a dull, leaden-gray color in their exposed fleshy parts, as compared with the light brownish or olive shade of normal animals.

Eggs laid by the submersed snails about Apr. 1, 1941, were placed on April 12 in containers made by removing the bottom from a small drinking glass and covering both ends with cloth netting (organdy for the earlier stages, mosquito netting for the older ones). These containers, suspended in a current of water maintained by aerators (see Fig. 1 C), provided better circulation of aerated water than the Petri dishes used in the first experiment. By May 31, at an age of about five weeks, the shell lengths of the young snails varied between 3 and 10 mm., only slightly less than those of snails of the same age in the regular laboratory cultures. In the hot weather of midsummer of 1941, when, in the absence of the writers the care of the cultures was entrusted to a laboratory assistant, the culture was lost. On July 29, 1941, about the time that the snails in the culture died, the temperature of the laboratory air reached 34.4° C., and the temperature of the cultures in the laboratory went above 30° C. (a reading at 8:30 a.m. on that date gave a temperature of 30.5° C.). Subsequent work by Dr. C. M. Vaughn (not yet published) has shown that, even in the case of snails allowed access to air in well-aerated laboratory cultures, temperatures of this magnitude result in some mortality.

The observations made in the course of these experiments on submersed snails show: (1) that *Lymnaea stagnalis appressa* can be grown through a complete life cycle in laboratory cultures at room temperatures without having access to the air; (2) that the growth of the snails under such conditions is somewhat retarded as compared with that of normal animals, but that in time they can reach maturity, lay viable eggs, and attain a size and life span only slightly less than that of normal snails.

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